Further Experimental Evidence concerning anti-TACI Antibodies of Example 18

Key

248.14.1.5.5 = 248.14 of EP1141274
248.23.1.3 = 248.23 of EP1141274
248.24.1.5.2 = 248.24 of EP1141274

Neutralization (Blocking) of Binding Assay by ELISA:

Chemiluminescent ELISA to determine the potential of the current cloning-stage Mabs to block the binding of zTNF4s-CF-BHK-biotin (10ng/ml) to TACI-BHK cells (BHK cells expressing TACI on its cell surface).

Experimental Design: Mab supernatants were offered to the TACI-BHK cells for binding. Then biotin-labelled zTNF4s-CF-BHK was offered to the TACI-BHK cells for binding. Visualized bound biotin-labelled zTNF4 with NeutaAvidin HRP.

Determined that supernatant from: 248.23.1.3 and 248.23.1.4 and 248.23.1.3.3 and 248.23.1.3.6 block binding of the biotin-labelled zTNF4 to TACI-BHK cells to a level of 4-fold over background.

FACS analysis of Reciprocal Blocking on TACI-Jurkat cells.

248.14.1.5.5 does bind to TACI-Jurkat cells already bound with labeled-zTNF4s-CF-BHK. But this Mab will completely inhibit the binding of 1ug/ml labeled-zTNF4s-CF-BHK to cells when offered as a competitor.

248.23.1.4 does not bind to TACI-Jurkat cells already bound with labeled-zTNF4s-CF-BHK. Presumably the antibody epitope is blocked or disabled by the bound zTNF4. But this Mab will block a small amount of zTNF4 binding when offered as a competitor.

248.24.1.5.2 does not bind to TACI-Jurkat cells already bound with labeled-zTNF4s-CF-BHK. Presumably the antibody epitope is blocked or disabled by the bound zTNF4. But this Mab will block a small amount of zTNF4 binding when offered as a competitor.

NB reference: Susan McMillen Notebook 7042 page 14.

FACS analysis of Mab binding on TACI-BHK and BCMA-BHK cells .

None of the 3 TACI Mabs demonstrates any binding to BCMA-transfected BHK cells.

NB reference: Susan McMillen, Notebook #7042, page 15

BiaCore analysis of Mab Epitope Competition:

The purpose of this experiment was to develop a screening assay for the next TACI fusion that would identify assay pairs of antibodies. By default, the assay would tell us if 2 Mabs occupy the same site or close to it. In using the current purified Mabs as guinea pigs, we were able to determine that 248.24 can capture TACI-MBP and subsequently bind 248.14. So we have a rudimentary assay pair.

From the experiment we also were able to determine qualitative affinities: 248.14 has the lowest affinity, 248.23 has intermediate affinity, and 248.24 has the highest affininty.

NB reference: Susan McMillen #7042 pages65-72